Further experiments show clearly that false plasmolysis plays a part in this process, for hypotonic solutions or even tap water or distilled water may produce a contraction of the inner surface while the turgidity of the outer surface is maintained.

The chromatophores are numerous and lie embedded between the inner and outer surfaces of the protoplasmic sack. They contain chlorophyll and likewise a red pigment which is soluble in water. The red pigment is unable to escape from the chromatophore into the protoplasm under normal conditions because the surface of the chromatophore is impermeable to it. When the separation of the inner and outer surfaces of the protoplasm reaches a certain point the surface of the chromatophores usually becomes permeable to the red pigment so that it diffuses out. The cells then present a very striking appearance. The contracted vacuole remains colorless while all the space between the inner and outer surfaces of the protoplasm becomes deep red. The red pigment can not escape through the outer surface, nor can it pass through the inner surface into the vacuole. The cell may remain in this condition for an hour or two. Finally the red color begins quite suddenly to diffuse through both the protoplasmic surfaces.

The nuclei behave as though their surfaces were impermeable to the red pigment at the start, but they appear to become permeable to it soon after it begins to diffuse out from the chromatophores.

The cell wall which encloses the protoplasm is freely permeable to the red pigment and to salts at all times, but is quite impermeable to many other substances.

Similar effects have been observed in a variety of other cells.

Whether these effects are due to true or to false plasmolysis or to a combination of both, it is evident that the various kinds of surfaces (*i. e.*, the inner and outer protoplasmic surfaces, and those of the chromatophores, of the nuclei and the cell walls) can be proven to differ greatly in their behavior with respect to permeability.

The term differential permeability may be suggested as an appropriate designation of these phenomena.

The conception of differential permeability may perhaps be extended to surfaces other than those described here. Since the protoplasm is composed of a variety of structures (down to those which are ultramicroscopic) and each of these has a surface it is quite possible that many kinds of semi-permeable surfaces exist within the cell.

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THE SOCIETY OF AMERICAN BACTERIOLOGISTS. II

SANITARY BACTERIOLOGY

Observations upon the Bacteriology of the Baltimore City Water in Relation to the Typhoid Fever Present, and the Effect of the Hypochlorite Treatment: WILLIAM W. FORD and ERNEST M. WATSON.

Since October, 1910, up to the present time (December, 1912), a period of a little over two years, it has been possible for us to follow the bacteriological condition of the Baltimore city water by systematic examinations (weekly)—excepting for a brief period in the summer of 1911. These examinations have been of the nature of the bacterial count, the determination of the number of fermenting organisms present by means of the Smith tube, the isolation and determination of the various species present. The purpose of this work was (1) to determine the relation, if any, between the extent of the pollution and the amount of typhoid fever in the city, (2) to determine the seasonal variations in the bacterial content of the water and (3) to ascertain the effect of alum and hypochlorite of lime upon the city drinking water, as regards the bacterial content and later the effect of the purity or pollution of the water under these conditions upon the amount of typhoid fever in the city. It was found that in 1910 and 1911 there was a striking relation between the period of summer and fall pollution of the water and the summer rise in the amount of typhoid fever. The number of organisms in the water at this time ranged from 1,000 to 5,000 per cubic centimeter, and fermentation took place in 1/10 to 1/100 c.c.

and on one occasion in 1/1000 c.c. The entire significance of this relation will be fully determined only by further study. It further was found that the bacterial content of the city water during periods of pollution was different from that during periods of relative purity. *Bacillus coli* comprised 55 per cent. of all organisms isolated during the period of relative purity, while during the period of pollution it comprised only 25 per cent. of organisms present. At this latter time, however, several new forms made their appearance, such as *lactis aerogenes*, intermediate group, attenuated forms and "liquefying fermenters." During little more than a year now it has been possible to observe the effect of hypochlorite of lime and later of alum on the bacterial flora of the city water. In the main the bacterial count has been greatly reduced under the chemical treatment, the counts practically always being less than 500 organisms per cubic centimeter. Notwithstanding this, however, the degree of fermentation has remained practically the same, *i. e.*, 1/10 and 1/100 c.c. of the water giving positive tests in the Smith tube. The typhoid fever during this period of chemical treatment has been slightly reduced. However, the reduction was not at all striking, which makes us believe that perhaps the greater part of our typhoid fever may not be water-borne or, on the other hand, if water-borne, the specific organisms of pollution have not been removed from the water by the chemical treatment in the usual strengths of available chlorine.

Some Results of the Hypochlorite Disinfection of the Baltimore City Water Supply: J. Bosley Tomas and Edgar A. Sandman, Baltimore City Water Department.

Stokes and Hachtel[1] have reported the result obtained by the hypochlorite disinfection of the Baltimore city water supply during a period extending from the institution of the treatment on June 15, 1911, to October 30, 1911. They examined samples taken from the untreated water in the impounding reservoir and from the treated water after it had passed through each of two storage reservoirs. The result of their examination showed bacterial reduction varying between 94.5 and 99 per cent. They also showed average reduction in the colon bacillus from 57.5 per cent. positive tests with 0.1 c.c. of untreated water to 12 per cent. positive tests with 0.1 c.c. of treated water, and from 89 to 40 per cent. with 1 c.c. The greatest reductions were obtained with one part per million of available chlorin, when there were shown reductions from 86 per cent. positive tests with 0.1 c.c. of treated water, and from 100 per cent. to 37 per cent. with 1 c.c. The period covered by the following report extends from January to December, 1912. In addition to the places sampled by Stokes and Hachtel we obtained samples at the influent of the first storage reservoir, after the water had passed through seven miles of tunnel subsequent to treatment. The time required for the water to pass through this tunnel varies between 4.9 and 12.2 hours. While allowing sufficient time for effective disinfection, the taking of samples just before the water enters the first storage reservoir permits of counts being obtained before any after-growths are likely to have occurred. The amount of available chlorin applied during the period covered by the report of Stokes and Hachtel was raised from 0.4 parts per million applied at the start on June 15, to 0.6 on June 23 and to 1 on October 15. On July 15, 1912, the amount was again raised, by order of the Commissioner of Health, to 1.5 parts per million, and this amount has been maintained until the present time. From January 11 to November 12 aluminum sulfate, in amounts varying between 0.610 and 1.05 grains per gallon, was applied to the water as it entered the first storage reservoir. Shortly after the period covered by the report of Stokes and Hachtel after-growths in the storage reservoir caused excessive bacterial counts. These conditions maintained during the first five months of the year, but about the middle of May the counts showed a marked diminution, and no further after-growths were observed, excepting during the few days in September. The monthly averages of the results in bacterial counts and *B. coli* tests, shown in the accompanying table, are taken from daily analyses. The counts during the first six months were obtained on standard agar at 20°, and during the remainder of the year at 37°. The *B. coli* averages were obtained from tests made on portions of water varying by a multiple of ten from 0.001 c.c. to 100 c.c., sufficient number of tubes being used in each case to secure at least one negative and one positive test, excepting when no fermentation was obtained with 100 c.c. The average number of *B. coli* per cubic centimeter for each month was estimated by considering the number of positive and negative tests in each dilution and following the method described by Phelps before the American Public Health Association in 1907. Lactose bile was used as an

[1] *Am. Jour. Pub. Health*, April, 1912.

initial medium, and Endo's agar was used for isolating the members of the *B. coli* group in pure culture, nearly 100 per cent. successful isolations having been obtained by the use of this medium, whereas the frequent encountering of spreaders on litmus agar and the fact that many of the acid-forming colonies proved not to be members of the colon group seriously impaired the efficiency of this latter medium. No attempt was made until in the last two or three months to differentiate the four members of the colon group; but this is now being done with the use of dulcit, in addition to the usual sugars, and morphological examinations, and the results seem to show a greater vulnerability of the two *B. coli* organisms than of *B. aerogenes* and *B. acidi lactici*. The results obtained by the use of the 20° temperature show much greater reduction in the bacterial count than those obtained with the 37° temperature, and we believe that counts should be made at the higher temperature in addition to those made at 20°. The effects of the treatment of this water supply have been a very good reduction in the bacterial count of the water as it enters the first storage reservoir, and almost entire elimination of the members of the *B. coli* group, the treated water during three months showing none of these organisms at any time in 100 c.c. The reduction in the number of cases of typhoid fever occurring in Baltimore during 1912 is 31 per cent., compared with an average of the number of cases occurring during the years from 1906 to 1910, and 24 per cent., compared with the number of cases occurring during 1911, in the last six months of which the water supply was treated. We wish to acknowledge indebtedness to Mr. Ezra B. Whitman, water engineer, and to Mr. Emory Sudler, engineer in charge of the improvement of the water supply, for an interest unusual with the engineers not directly acquainted with the details of the laboratory work.

Experimental Disinfection of Water with Calcium Hypochlorite (preliminary note): F. W. HACHTEL, M.D., and RAYMOND FREAS, A.B., Bacteriological Laboratory of the State and City Boards of Health, Baltimore, Md.

The following brief report is made upon certain experiments that were begun in the midsummer of 1911 and which have for their object the determination of the amount of available chlorine necessary to eliminate the *B. coli* from 10 c.c. of water under varying conditions of turbidity. They were instituted because, although the quantity of available chlorine added to the Baltimore drinking water had gradually been increased from 0.4 to 0.75 parts to the million gallons, the colon bacillus still persisted with too great frequency in 1 and 10 c.c. of water collected at the storage reservoirs and as drawn from the taps. The first series of experiments had to be done in a hurry, as heavy rains on the watershed were markedly increasing the turbidity. At this time, therefore, only presumptive tests in lactose bile were done. This, however, is not a guide to the sanitary condition of water treated with hypochlorite of calcium as shown by some work carried on in the laboratory on samples collected from the taps and storage reservoirs after treatment. As a result of these we found that although they would not infrequently produce gas in lactose bile, we were unable to obtain the colon bacillus in pure culture even after repeated platings in lactose-litmus-agar. A number of these were then plated out anaerobically and in a considerable percentage of cases we obtained *B. welchii* or the *B. sporogenes* or both. This, therefore, led us to repeat our work, and the results may be summarized as follows: With a turbidity of 32 we have found that 0.75 and 1 part of available chlorine to the million gallons caused a bacterial reduction of about 80 and 90 per cent., respectively, in six hours. During the same period the bacterial content of the untreated water was doubled. At the end of twenty-four hours, although the untreated water showed a count of 300 times as great as when the experiment was started, the two treated waters gave counts of only 0.3 per cent., as great as that of the raw water at the beginning. Again, the water before treatment contained the colon bacillus in 1 c.c. but not in 0.1 c.c.; at the end of one hour after the addition of calcium hypochlorite in the aforementioned quantities 1 c.c. of the treated water failed to ferment. In addition, although there was gas formation in all the lactose-bile tubes inoculated with 10 c.c. for the first six hours after treatment, nevertheless the colon bacillus was not isolated from any of these, though they were plated out on three consecutive days. On the other hand, we were able to obtain *B. welchii* or *B. sporogenes* from almost all of them. Four, five, six and twenty-four hours after the addition of hypochlorite 50 c.c. of each of the treated waters were inoculated into large tubes of lactose-bile and although fermentation occurred in all save one, *B. coli* was not obtained from any of these in spite of repeated attempts. In every

instance but one *B. welchii* was present. It should be stated that the water was kept in the dark and at the out-of-door temperature. The calcium hypochlorite used had 34.1 per cent. of available chlorine. It is worth noting that in one experiment with water of a turbidity of 12 in which we used 0.75 part of chlorine per million the colon bacillus was present in 1 c.c. at the end of two hours and in 10 c.c. at the end of three hours. In addition to this, 10 c.c. of the treated water still caused fermentation at the end of six hours, but not after twenty-four hours. The colon bacillus, however, was not isolated from any of these. In this instance the bacterial content of the water was about one seventh as great as in the previously described case. This result is to be ascribed to the very low available chlorine content of the hypochlorite used —this being only 1.546 per cent. We have been unable to repeat the experiments with water of very high turbidity, owing to the lack of heavy rains on either of the two watersheds, but we purpose to do so at the first opportunity. Besides this we propose to determine if there is any relation between the temperature of the water treated and the amount of chlorine necessary to destroy *B. coli*. So far we can but state that, with water of a turbidity of about 30, a bacterial content of 15,000 and the colon bacillus present in 1 c.c. and not present in 0.1 c.c., 0.75 part of chlorine to the million gallons eliminates *B. coli* from 10 c.c. in one hour and from 50 c.c. certainly in four hours and possibly in less time; of course this presupposes the use of hypochlorite of high available chlorine content.

The Distribution of B. coli in Polluted Oysters: John W. M. Bunker, Ph.D., instructor in sanitary analysis, Harvard University.

To establish whether the distribution of *B. coli* in polluted oysters is or is not uniform throughout the regions of the oyster body, examination was made of the following regions of 145 oysters taken from regions subject to varying conditions of pollution in Narragansett Bay: shell liquor from the branchial chamber, material from the mouth, material from the stomach, material from the intestine at the point where it bends sharply upon itself, material from the extremity of the intestine, shell liquor from the cloacal chamber, decanted mixed shell liquor. As a result of these examinations it is evident that (1) the distribution of the colon bacillus is not uniform throughout the various regions of a polluted oyster; (2) of the body regions, the stomach, in general, contains the colon bacillus most frequently; (3) at all seasons of the year the colon bacillus is found more frequently in the shell liquor than in any portion of the body; (4) when the temperature of the water on the oyster beds is below from 6°to 8° C., the best index of pollution as afforded by the *B. coli* test can be obtained from the liquor in the cloacal chamber; (5) at temperatures of above 8° C. the liquor in the branchial chamber is the most reliable source of information regarding pollution; (6) at no season of the year does the practise of decanting the shell liquor afford the most reliable index of pollution that could be obtained.

The Bacteriology of the Hen's Egg: Leo F. Rettger, Sheffield Scientific School, Yale University.

In our investigations of bacillary white diarrhea in chicks we have made bacteriological examinations of at least ten thousand eggs. While our chief object was the detection of *B. pullorum*, the specific cause of the disease, a general bacteriological study was made of the eggs, and particularly those which were fresh and apparently normal. Until the spring of 1912 the yolks only were examined, as a rule. During the past year special tests were made with the whites. In the examination of the yolks of fresh and unincubated eggs the entire yolks were employed. They were removed aseptically and mixed in special test tubes of large diameter with 25 cubic centimeters of plain bouillon. The tubes were kept three to four days at 37° C., and for an additional period of twenty-four hours at 20°. Streaks were made with platinum loops on slant agar. Incubated eggs were tested directly, that is a small amount of the yolk was streaked over the surface of slant agar. In the testing of whites 5 cubic centimeters of the egg-white were mixed with 100 c.c. of sterile tap water. These tests were made in duplicate. One flask of the diluted white was kept for five to six days at 20° and the other at 37°. Slant agar streaks were then made. From the results of the numerous tests we were led to conclude that the yolks and whites of fresh eggs were, as a rule, sterile. Among the organisms found (aside from *B. pullorum*) the most conspicuous was a large spore-bearing bacillus, resembling in many ways *B. mesentericus*. In addition to this the following were observed: *Proteus vulgaris, B. pyocyaneus, B. fluorescens, B. coli,* cocci and moulds. It is quite probable that many of the organisms obtained in the tests were contamination forms. Eggs which were incubated artificially for from

one to three weeks seldom gave us any indications of containing bacteria. The only organism which could be regarded without doubt as coming from the interior of the egg was *B. pullorum*, and this was always found, when present, in the yolks of both fresh and incubated eggs. The results have been quite different, however, with eggs that were kept in warm, damp places for any length of time, and those which were left for several days under sitting hens. Such eggs, especially the infertile, frequently contained bacteria.

On Antiseptic and Bactericidal Properties of Egg White: JOEL A. SPERRY, 2d, M.S.

The white of the eggs was aseptically transferred to sterile test tubes in 5 cubic centimeter quantities and then inoculated with various organisms. Small amounts of the egg white were introduced into dilution flasks and agar plates were made with 0.5 c.c. of the dilution. The egg white showed strong bactericidal properties toward *Subtilis cereus* and *megatherium* while towards *coli, typhi, anthrax, Proteus vulgarus, Staphylococcus pyogenes aureus* and other organisms the antiseptic action only was noticeable. This was true for the white of fresh eggs and cold-storage eggs not more than nine months old. The action of egg white on *putrificus*, malignant edema and symptomatic anthrax seemed to be purely antiseptic. The white of eggs which are eleven months old or more showed a tendency to lose these properties.

SOIL BACTERIOLOGY

A New Method for the Bacteriological Examination of Soils: P. E. BROWN, Iowa State College, Ames, Iowa.

A brief statement of the situation regarding the bacteriological examination of the soils brings out as salient points that the mere quantitative examination of soils is of little value from the fertility standpoint; that the logical means by which conclusions can be reached concerning the influence of varying bacterial content on crop production consists of certain groups of organisms as measured by the chemical products of their growth and actual crop production; and that a necessity therefore for progress in the work is the formulation of satisfactory methods for measuring the activities of certain important groups of soil organisms. A discussion of the methods previously employed while recognizing certain value attached to the results obtained thereby, points out the objections to the solution method and to the use of sterilized or air-dry soil as media and the conclusion is reached that fresh soil is the logical medium to be employed. Plots differentiated through special treatment were employed in experiments and satisfactory results were secured using fresh soil with ammonium sulfate for nitrification and fresh soil with mannite for azofication. For ammonification more difficulty was experienced in selecting a suitable nitrogenous material to permit of an accumulation of ammonia in sufficient amounts to be measured. Comparisons of the results obtained using air-dry soil and infusions of fresh soil and dried blood, albumen and casein with those secured using fresh soil and the same nitrogenous materials showed that casein added in solution to fresh soil brought out the greatest differences in the ammonifying power of the soils and possessed also certain other advantages incident to manipulation. The method recommended consists then in testing of fresh soil obtained as described in previous work by the writer, adding a solution of casein for ammonification, ammonium sulfate for nitrification and mannite for nitrogen fixation.

A Cultural and Morphological Study of some Azotobacter: DAN H. JONES, Ontario Agricultural College, Canada.

From various samples of soil taken from the garden of the Ontario Agricultural College, sixteen colonies of *Azotobacter* were isolated. A study of these cultures extending over two years shows them to comprise four distinct varieties or species. These have been tentatively named *A*1, *A*2, *A*3 and *A*4. *A*1 and *A*2 bear a resemblance to *Azotobacter chroococcum* and *A*3 and *A*4 bear a resemblance to *Azotobacter agilis*, as described by Beyerinck. All cultures in Ashby's solution fix atmospheric nitrogen in the form of nitrates. In young cultures (one to two days old) of each variety, the organism is a short, thick rod with rounded ends, frequently occurring in diplo form and motile by means of peritrichic flagella. At this stage, the internal protoplasm is homogeneous, though occasionally what may be a nucleus in the form of a spherical granule is present, this undergoing a fission when the cell divides. When cultures are four to five days old, the cells become irregularly spherical, coarsely granular and nonmotile. The granules enclosed are spherical, vary in size and number and are often of two kinds. The one kind of granule gives the glycogen reaction when treated with iodine-potassium-iodide solution, but is negative to certain anilin dyes, whereas the other kind is negative to the glycogen

stain but positive to the anilin dyes. This second kind of granules appear to arise from the aforementioned nuclear body, and the first mentioned kind appear to be a product of the cell activities, possibly a reserve food supply. In active cultures from five to ten days old, many of the cells disintegrate, their enclosed granules being scattered. The granules of the second type appear to give rise to new organisms, acting in this particular as gonidia, while those of the first type slowly disappear as though they were dissolved. At this stage, *A*1 and *A*2 produce large capsules, but *A*3 and *A*4 do not. Fission frequently takes place within these capsules, thus producing an irregular group of from two to six or more organisms within a capsule. When cultures are about three weeks old, the majority of the organisms appear as spheres, *A*1 and *A*2 in irregular clusters, *A*3 and *A*4 in fairly regular packet and sarcinæ forms. This condition occurs only when the cultures are near their full development and appears to be a resting stage. Chains of from four to thirty cells are common in old liquid cultures as Ashby's solution. Involution forms of a great variety in size and shape appear in old cultures, but the most striking changes in morphology occur in cultures incubated at 37° C., especially in case of *A*1, in which many of the cells elongate into tubes 40 or 50 μ long. Colonies and streak cultures on Ashby's agar are first hyaline, then white and when they are fully developed a brown pigment is produced, which in case of *A*2, *A*3 and *A*4 in time frequently becomes black. Mass cultures of *A*1 are very moist and have a tendency to flow; those of *A*2, while being moist, do not flow, but become contoured in topography; those of *A*3 are pasty; those of *A*4 somewhat coriaceous and verrucose. Ashby's media give the best growth, beef extract media allowing but a restricted development. Good growth on Loeffler's blood serum.

The Origin of Certain Organic Soil Constituents: M. X. Sullivan.

Examination was made of the dried mold, *Penicillium glaucum*, grown on Raulin's solution and of the filtered solution after mold growth for organic constituents. In the alcoholic soda extract of the mold were found oleic and palmitic acids and a fatty acid melting at 54° C., hypoxanthine, guanine, and adenine, histidine, thymine, choline, probably lysine and a small amount of hydroxy-fatty acids. In the direct alcohol extract was found mannite, cholesterol bodies, hypoxanthine and cerebroside. In the culture solution were found fatty acids, guanine, adenine and hypoxanthine, a small quantity of histidine, pentose sugar, unidentified aldehydes and a small amount of hydroxy-fatty acids. Most of these compounds have been found in soil and the conclusion is made that in the formation of the various organic soil constitutents, microorganisms, such as yeasts, bacteria and molds, play an important part.

Soil Inoculation under Soil Conditions of Lime Deficiency: T. D. Beckwith.

The Cascades divide the state of Oregon roughly into two sections differing greatly as to rainfall and consequent seepage of soluble soil constituents. Much of the land in the Willamette Valley and western section of the state has a lime deficiency of from one to five tons per acre-foot. With the idea of learning whether or not artificial inoculation of legume seed with pure cultures of *B. radicicola* might be expected to yield results, reports of success or failure of soil inoculation cultures furnished by the department of bacteriology have been sent to Oregon Agricultural College, accompanied by root specimens. During the past summer 110 tests have been carried out, at least 60 of which have been with alfalfa. A compilation of the results obtained shows that the method was beneficial in 69 per cent. of the experiments. On the contrary, of 50 tests carried out in the eastern part of the state in soils well furnished with lime, success was obtained in 45 instances, or 90 per cent. It is thus evident that *B. radicicola* may retain virulence to the roots of legume plants, under conditions of a small amount of soil acidity. Results were unfavorable when lime deficiency was over five tons per acre-foot.

Bacterial Activity in Soil as a Function on the Various Physical Soil Properties: Otto Rahn, University of Illinois.

To study the influences of physical soil properties upon bacterial activity in soil, pure cultures of *B. mycoides* in quartz-sand peptone water mixtures were studied. In one series, cellulose was added to the sand. The amount of ammonia formed under these conditions was taken as the indicator of bacterial activity. Further, *Bact. lactis acidi* was grown in milk sand mixtures, acidity and number of cells serving as measure of development. The conclusions are greatly influenced by the basis of comparison. If the data are computed per 100 g. of dry soil, as is customary among soil bacteriologists, it would seem that the bacteria thrive best in a fairly moist sand (20–25 per cent.). If, however, the actual culture

medium, *i. e.*, the peptone solution, is used as basis, ammonification is most rapid in the driest soil (10 per cent. water). If the data are computed per 100 c.c. of soil solution, the concentration of the solution is again of greatest importance. The results vary greatly if one time the peptone is given in proportion to the amount of soil and another time in proportion to the amount of soil moisture. The farmer is primarily interested in the amount of plant food per weight of soil; the efficiency of bacteria can be determined only by comparing equal amounts of culture medium and of food. In test tube or flask cultures of *B. mycoides,* oxygen is always in the minimum. In sand cultures, the oxygen exchange is greatly increased and the rate of development is correspondingly higher. The oxygen exchange between gas and liquid depends upon the oxygen content of the soil air and upon the surface exposed to this air. The surface per unit of liquid is inversely proportional to the diameter of the soil particles and to the moisture content of the soil. The oxygen content of the soil air depends upon the ventilation which is nearly proportional to the square of the grain diameter. A thinner film of moisture gives therefore a faster decomposition, but there is a limit to the thinness of this film, extremely thin films causing a retarded decomposition. The optimum thickness of moisture film in the case of *B. mycoides* was between 20 and 40 microns. This film was obtained in sand of 1 mm. diameter at a moisture content of about 10 per cent. In arable soils, with a grain size not more than 0.1 mm., it would require more than 50 per cent. of moisture to produce the optimum film thickness. In other words, strictly aerobic bacteria will never find optimum conditions of existence in soils. The ultimate endpoint of decomposition, if the food concentration was constant, was the same in the case of *B. mycoides,* since only the rate of decomposition was influenced by the efficiency of the oxygen supply. With some other bacteria, the endpoint varied greatly. The behavior of anaerobic bacteria, represented by *Bact. lactis acidi,* was in accordance with the above-mentioned principles, the main factor for their development being a very thick moisture film. The physical effects of undecomposed organic matter were imitated by the addition of finely ground filter paper to sand. In fairly dry soils, cellulose caused a decrease of ammonia formation by making some of the soil moisture unavailable for bacteria. In the moisture sands, cellulose increased the ammonification probably by holding the sand particles farther apart and thus increasing aeration.

Characteristics of Cellulose-destroying Bacteria: I. G. McBETH, F. M. SCALES and N. R. SMITH.

Seventeen species of cellulose-destroying bacteria have been isolated and studied; 7 of these belong to the genus *Bacillus,* 4 to the genus *Bacterium* and 6 to the genus *Pseudomonas.* All are morphologically and physiologically different from Omelianski's hydrogen and methane ferments. None of the species studied have shown any tendency to form gaseous products, and in relation to oxygen all are facultative aerobes. By means of cellulose agar colonies the species may be separated into two distinct groups: those forming opaque colonies which clear a well-defined zone beyond the colony and those which form transparent colonies with little or no indication of an enzymic zone. All of the organisms grow more or less rapidly on beef gelatin, but only 10 of the 17 species studied have shown any power to liquefy gelatin. On beef agar 11 species grow rapidly and luxuriantly, 4 species grow poorly and 2 have failed to give any growth at all. When introduced into Dunham's solution 9 species have shown the power to form ammonia. The action on litmus milk is also quite variable; 10 species give an acid reaction, 5 an alkaline reaction and 2 make no growth. The digestion of the milk occurred with only 4 species. Eleven species have shown a growth on potato cylinders while 6 have shown no growth or only a slight bleaching action along the track of the inoculum. The action of the cellulose-destroying bacteria studied shows marked differences in their activity toward the other carbohydrates such as dextrose, lactose, maltose, saccharose, glycerine, mannite and starch in peptone solutions. In their relation to these solutions the cellulose-destroying organisms may be divided into the following groups: (1) those which give an acid reaction from all of seven peptone carbohydrate solutions used; (2) those which give an alkaline reaction from all of the peptone carbohydrate solutions; (3) those which give an acid reaction from only a part of the peptone carbohydrate solutions; (4) those which produce no change in the reaction of any of the peptone carbohydrate solutions.

A Plan for Revivifying Bacteria by Groups: H. J. CONN.

Our present standard method of revivification, in non-saccharine broth at 37°, is not applicable

to most soil organisms nor to many other bacteria. A possible standard method is here suggested for revivifying the bacteria that do not grow under such conditions. The bacteria are to be divided into five groups:

1. Growing well in plain broth at 37° C.
2. Excluded from group 1, but growing well in plain broth at 20° C.
3. Excluded from groups 1 and 2, but growing well in dextrose broth at 37° C.
4. Excluded from groups 1, 2 and 3, but growing well in dextrose broth at 20° C.
5. Excluded from all four groups, but growing well on surface of agar.

Each of these groups is to have its own method of revivification, as follows: 1, in plain broth at 37° (as at present); 2, in plain broth at 20°; 3, in dextrose broth at 37°; 4, in dextrose broth at 20°; 5, on agar slants. This classification includes most soil bacteria and many others; but further groups may be added as they prove necessary. These groups are somewhat similar to the groups of the bases recognized by chemists in qualitative analysis. Like the chemical groups, they are to be disregarded after the unknown has been determined.

The Ammonifying Efficiency and Algal Content of Certain Colorado Soils: Walter G. Sackett.

The power to transform organic nitrogen into ammonia is a property common to many cultivated Colorado soils. Soils in the incipient stage of the niter trouble appear to surpass our normal soils in ammonifying efficiency. Compared with soils from other localities, our niter soils excel in ammonifying efficiency to a very marked degree. Nineteen of the thirty-one soils examined have ammonified cottonseed meal more readily than the other nitrogenous materials employed; the remaining twelve have broken down in the dried blood most easily; twenty-six have formed ammonia from alfalfa meal more readily than from flaxseed meal, and with five the reverse has been true. The maximum per cent. of ammonia produced in seven days by any soil from 100 mg. of nitrogen as cottonseed meal was 51.98 per cent.; as dried blood 52.64 per cent.; as alfalfa meal 34.85 per cent.; as flaxseed meal 12.15 per cent. Algæ occur abundantly in many cultivated soils of Colorado. Twenty-one different species of algæ were found in the soils examined. With but two exceptions, all the species found belong to the blue-green algæ (Cyanophyceæ.) The family Nostocaceæ is best represented. There is a predominance of forms possessing thick, gelatinous sheaths. This paper is published in full as Bulletin 184 of the Colorado Experiment Station, Fort Collins, Colorado.

Nitrogen Fixation by Organisms from Utah Soils: E. G. Peterson and E. Mohr.

This paper is a preliminary note in a proposed extensive investigation regarding the fixation of nitrogen in Utah soils and the rôle played by microorganisms in this action, together with the various agencies influencing bacterial action. Samples of soil from which the organisms described were isolated were taken weekly from January 9 to November 4, 1912, from Greenville Experiment Farm, Utah Experiment Station. 100 c.c. portions of mannite solution were inoculated with 10 grams of soil and incubated at 20° C. After ten days' incubation subcultures were made in mannite solution and incubated for ten days at 20° C. Isolations were made from plates which were made from these subcultures. Several types of colonies were formed, but only three appeared that grew readily and for a long period on mannite agar. The paper describes these three forms. One of the three forms was undoubtedly *Azotobacter chroococcum,* the other two heretofore undescribed in western soils. Type No. 1 fixed 5.335 mg. of nitrogen in twenty days in mannite solution, average of 15 tests; type 2 (*Azotobacter chroococcum*) fixed 5.616 mg. of nitrogen in twenty days, average of 10 tests; type No. 3 fixed 5.588 mg. of nitrogen in twenty days, average of 12 tests. Analyses were made from January 9 to October 28 to determine if possible any marked seasonal variations in nitrogen fixation. The technique involved the addition of definite quantities of soil, taken under standard conditions, to mannite solution, the amount of nitrogen in the soil being subtracted from the amount of nitrogen present at the end of twenty days in order to determine the amount fixed. The variation was found to be very marked from week to week without apparent regularity, a marked increase in fixation power being noted from the middle of May to the end of June. Isolations were made from these impure cultures to determine the presence of the three colony types described in the paper. Types No. 1 and 3 were present in the majority of samples, type No. 1 predominating in all cases. Type No. 2 was present once in April, twice in June and once in September. Further work is being done on the three forms isolated.

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Secretary

(*To be concluded*)